methacrylamidopropyl) trimethylammonium (PMAETMA) maleimide, and poly(-lysine) (PLL) maleimide, poly(acrylic acid) (PAA) maleimide, poly(styrene sulfonate) (PSS) maleimide, poly(acrylic acid-stat-dimethylaminoethyl methacrylamide) (P(AA-stat-DMAEMA)) maleimide), and poly(arginine methacrylate) maleimide), or derivatives thereof; maleimide containing biopolymers, such as gelatin maleimide, cellulose maleimide, hyaluronic acid maleimide and alginate maleimide; and maleimide containing nucleobase polymers (i.e., maleimide containing polymers of adenine, thymine, guanine and/or cytosine repeating units); or any combination thereof.

[0018] In an embodiment, the maleimide containing polymer comprises a poly(ethylene glycol) (PEG) maleimide.

[0019] In an embodiment, the maleimide containing polymer comprises a gelatin maleimide.

[0020] In an embodiment, the bis-thiol containing cross-linking agent is selected from synthetic polymers, biopolymers, small molecules, bioactive molecules, or any combination thereof.

[0021] In an embodiment, the synthetic polymer is selected from PEG-bis-thiols, poly(N-isopropylacrylamide)-bis-thiols (NIPAAM-bis-thiols), poly(acrylic acid)-bis-thiols, poly(methacrylic acid)-bis-thiols, poly(styrene sulfonate)-bis-thiols, poly(amide)-bis-thiols, or any combination thereof. In an embodiment, the bis-thiol containing cross-linking agent is a PEG-bis-thiol.

[0022] In an embodiment, the biopolymers are selected from thiol-gelatin, thiol-cellulose, thiol-chitosan, thiol-hyaluronic acid, or any combination thereof.

[0023] In an embodiment, the small molecule is dithiothreitol (DTT).

[0024] In an embodiment, the bioactive molecules are selected from short chain peptides with at least two cysteine amino acid groups, inert short chain peptides, enzyme responsive short chain peptides, matrix metalloproteinase (MMP) responsive peptides, or any combination thereof.

[0025] In an embodiment, the bis-thiol containing bioactive molecule is selected from an MMP-responsive bis-cysteine peptide.

[0026] In an embodiment, the MMP-responsive peptide may be GCIPVSLRSGCG, GCRDGPQGIWGQDRCG, GCRDPLGLDRCG, GCRDEAPLKQDRCG, or any combination thereof.

[0027] In an embodiment, the maleimide containing polymer is PEG-maleimide and the bis-thiol containing cross-linking agent is selected from the group consisting of PEG-bis-thiol, an MMP-responsive peptide, and a combination thereof.

[0028] In an embodiment, the maleimide containing polymer is PEG-maleimide substituted with at least one bioactive molecule selected from RGD, YIGSR and IKVAV and the bis-thiol containing cross-linking agent is selected from PEG-bis-thiol, an MMP-responsive peptide, or a combination thereof.

[0029] In an embodiment, the MMP-responsive peptide is GCIPVSLRSGCG, GCRDPLGLDRCG, or a combination thereof.

[0030] In an embodiment, the maleimide containing polymer is gelatin maleimide and the bis-thiol containing cross-linking agent is PEG-bis-thiol.

[0031] In an embodiment, the maleimide containing polymer is gelatin maleimide and the bis-thiol containing cross-linking agent is PEG-bis-thiol.

[0032] In an embodiment, the molar ratio of maleimide containing polymer to bis-thiol containing polymer is in the range of about 10:1 to about 1:10. The ratio of thiol groups in the bis-thiol containing cross-linker(s) to maleimide groups in the maleimide containing polymer(s) may be greater than about 60%.

[0033] The hydrogel may be formed by printing a drop of polymer bio-ink onto a substrate followed by a drop of activator to form a hydrogel droplet, or a drop of activator can be applied to the substrate followed by a drop of polymer bio-ink to form the hydrogel. Repeating the printing steps forms a hydrogel. During the printing process cells can be included with the polymer bio-ink, the activator, or both polymer bio-ink and activator. Additionally or alternatively, cells may be included with a cell culture medium. The cell culture medium may be included with the polymer bio-ink, the activator, or it may be separate from the polymer bio-ink and the activator. For example, the cell culture medium may be suspended between layers of 3D printed hydrogel formed from the polymer bio-ink and the activator or it may be deposited on a surface of the hydrogel.

[0034] In an embodiment, the substrate is selected from any suitable vessel. Examples include microtitre plate of different well configuration (6, 24, 48 and 96-well), microtitre plate with coverslip bottom of different well configuration (6, 24, 48 and 96-well), fluorodish of various sizes, chamber slides of different chamber configuration (1, 2, 4, 8 and 16), cover slip or microscope slides. The vessel may be suitable for containing, holding or growing cells.

[0035] In an embodiment, the 3D printed hydrogel further comprises a bioactive molecule. The bioactive molecule may be bound to the maleimide containing polymer, the bis-thiol containing cross-linking agent, or both the maleimide containing polymer and the bis-thiol containing cross-linking agent. Additionally or alternatively, a free bioactive molecule may be present in the 3D printed hydrogel. The free bioactive molecule may be present in the polymer bio-ink, the activator, or both. The bioactive molecule may be selected from a peptide, MMP-responsive peptide, protein, polysaccharide, drug, therapeutic agent, antibody, small molecule inhibitor, kinase inhibitor, phosphatase inhibitor, antigen, pathogen, platelet, growth factor, cytokine, amino acid, nutrient, conditioned media, antibiotic, antiviral, RNA, and any combination thereof. Nanoparticles can also be incorporated into the 3D printed hydrogel.

[0036] In an embodiment, the bioactive molecule is selected from CRGDS, CIKVAV, CYIGSR, VEGF with C-terminal unpaired cysteine, protein (e.g., laminin, collagen), and MMP-responsive peptides (e.g., GCIPVSLRSGCG, GCRDGPQGIWGQDRCG, GCRDPLGLDRCG, GCRDEAPLKQDRCG); and any combination thereof.

[0037] In an embodiment, the 3D printed hydrogel further comprises a cell culture medium. Examples of suitable culture media include Dulbecco's Modified Eagle Medium (DMEM), Minimum Essential Media (MEM), Iscove's Modified Dulbecco's Medium (IMDM), Media 199, Ham's F10, Ham's F12, McCoy's 5A and Roswell Park Memorial Institute (RPMI) medium. The cell culture medium may be present in the polymer bio-ink, the activator, or both.

[0038] In an embodiment, the hydrogel contains cells.

[0039] In an embodiment, cells are present in the polymer bio-ink, in the activator, both in the polymer bio-ink and the